Arch Virol (2009) 154:1859–1860
DOI 10.1007/s00705-009-0508-4

ANNOTATED SEQUENCE RECORD

Complete nucleotide sequence of an isolate of *Euphorbia mosaic virus* that infects *Euphorbia heterophylla* and *Wissadula amplissima* in Jamaica

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Received: 11 May 2009 / Accepted: 26 August 2009 / Published online: 23 September 2009

Begomoviruses (family *Geminiviridae*) are whitefly-transmitted viral phytopathogens that devastate many cash and basic food crops worldwide [6]. *Euphorbia heterophylla* and *Wissadula amplissima* are widely distributed throughout Jamaica and oftentimes exhibit yellow mosaic and leaf curl symptoms. The agent that causes yellow mosaic of *E. heterophylla* in Jamaica has not been identified; however, partial sequences isolated from symptomatic *W. amplissima* suggest that it is infected by three distinct begomoviruses, one of which was tentatively named Wissadula golden mosaic St Elizabeth virus (WGMSEV) [1, 5]. In Mexico, *E. heterophylla* is infected by the begomovirus Euphorbia mosaic virus (EuMV) [3]. Here, we report that WGMSEV is actually an isolate of EuMV that infects both *E. heterophylla* and *W. amplissima* in Jamaica, and this isolate has genome features that distinguishes it from previously reported EuMV isolates.

In 2006, *W. amplissima* sample W96 [1], from which partial sequences of WGMSEV were isolated, and *E. heterophylla* exhibiting yellow mosaic were collected from Dunder Hill in the parish of St Elizabeth. Total DNA was extracted from the leaf tissues of these plants [4] and used as template in PCR. The partial sequences available for WGMSEV enabled the design of primers to amplify complete DNA-A and DNA-B (Supplementary data, Table 1). Amplicons were cloned and sequenced at Macrogen (South Korea). The Basic Local Alignment Search Tool (BLAST, http://www.ncbi.nlm.nih.gov/) was used for sequence similarity searches, whilst DNAStar (DNAStar Inc., Madison, WI, USA) was used for sequence alignments and phylogenetic inferences.

Full-length DNA-A clones pEAFL15 (FJ407052) and pW96AFL15 (DQ395342) were isolated from *E. heterophylla* and W96, respectively. These DNA-A sequences were 2,609 nt and 99% identical, suggesting that they are isolates of a single begomovirus. Clone pW96AFL15 was used as a representative DNA-A in subsequent analyses. The partial DNA-A sequence of WGMSEV [1] was 97% identical to the corresponding region of pW96AFL15, suggesting that pW96AFL15 could represent the complete DNA-A of WGMSEV. BLAST similarity searches showed that pW96AFL15 was most similar to Western Hemisphere begomoviruses and shared highest sequence identities with isolates of EuMV. Clone pW96AFL15 was 96% identical to an A strain of EuMV isolated from Jurabo, Puerto Rico, in 1991 (EuMV-A[PR:Jur:91]) and a B strain isolated from Jalasco, Mexico, in 2005 (EuMV-B[MX:Jal:05]). Additionally, pW96AFL15 was 92% identical to an A strain of EuMV isolated from Yucatan, Mexico, in 2004 (EuMV-A[MX:Yuc:04]). Begomoviruses sharing greater than 89% DNA-A sequence identity constitute a single species [2], suggesting that pW96AFL15 is an isolate of EuMV. The tentative species WGMSEV is therefore renamed EuMV-[Jamaica:StElizabeth:2006], abbreviated EuMV-[JM:SE:06]. The full-length DNA-B clone isolated from W96, pW96BFL1 (EU740969), was 2571 nt and is the likely DNA-B of EuMV-[JM:SE:06], as it shared a 165-nt CR of 96% identity with this DNA-A. Clone pW96BFL1

Electronic supplementary material The online version of this article (doi:10.1007/s00705-009-0508-4) contains supplementary material, which is available to authorized users.

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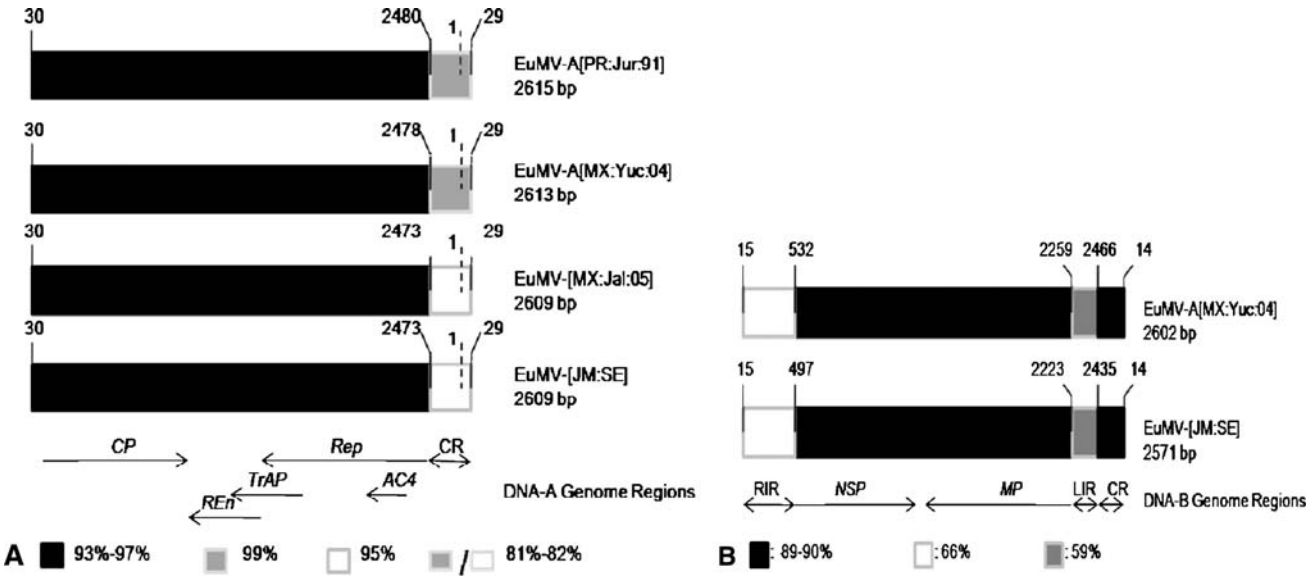

Fig. 1 Illustration of the genome divergence between different EuMV isolates. **a** DNA-A and **b** DNA-B. *RIR* right hypervariable intergenic region, *LIR* left hypervariable intergenic region

was most identical, 83%, to the DNA-B of EuMV-A[MX:Yuc:04]. Phylogenetic analyses placed EuMV-[JM:SE:06] in the squash leaf curl virus cluster, as reported for EuMV-[MX:Yuc:04] [3].

Comparison of all four EuMV isolates revealed that the DNA-A of EuMV-[JM:SE:06] and EuMV-B[MX:Jal:05] are highly congruent and diverge from the EuMV-A strain in size and CR identity (Fig. 1a). DNA-B is available for EuMV-[JM:SE:06] and EuMV-[MX:Yuc:04] only, and they differ mostly in their long non-coding left and right hypervariable intergenic regions (Fig. 1b). Comparison of the DNA-B of different strains within other begomovirus species revealed that the DNA-B of strains from different countries are <89% identical, whilst strains isolated from the same country have DNA-B identities >90% (Supplementary data, Table 2). The 83% identity between EuMV-[JM:SE:06] and EuMV-A[MX:Yuc:04] is in accord with the lower identities between DNA-B sequences of strains isolated from different countries.

The experimental host range of EuMV-[JM:SE:06] was investigated by biolistic inoculation using tandem repeats of its genome components (Supplementary data, Fig. 2), and it infected *Capsicum annum, Lycopersicon esculentum*, *N. benthamiana* and *Phaseolous vulgaris*, as was reported for EuMV-[MX:Yuc:04] [3]. In Mexico, EuMV infects peppers, and *E. heterophylla* serves as a natural reservoir from which EuMV can be disseminated to crops [3]. In Jamaica, both *E. heterophylla* and *W. amplissima* maintain EuMV and could facilitate its adaptation to crops.

Acknowledgments This work was funded by research grants from the University of the West Indies, Mona Campus, School of Graduate Studies and Research, the New Initiative Programme and from the office of the principal.

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